Courtship Behavior of the Dainty Sulfur Butterfly, *Nathalis iole* with a Description of a New, Faculative Male Display (Pieridae)

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Abstract. The components and temporal structure of courtship leading to copulation are described for the dainty sulfur, *Nathalis iole* (Boisduval). Most successful courtships were similar to those described in the literature for several other pierids. However, in 22% of the successful courtships the male performed a previously undescribed wing spread display in which he alit in front of and facing away from a perched female and assumed a stationary posture with his wings fully spread. This display is elicited by the performance of initial rejection responses by the female. The proximate and ultimate causes of this faculative male display are discussed.

Introduction

In butterflies, male courtship behavior is viewed as having evolved in response to mate choice by females to insure sex and species identity, and quality of a potential mate (Scott, 1972; Silberglied, 1977; Rutowski, 1982). Because errors in the selection of mates can have severe negative effects on a female's reproductive success, it is expected that all males successful in courtship should be required by females to produce basically similar performances. While collecting data for another study, observations on the courtship of the dainty sulfur, *Nathalis iole* Boisduval, revealed the existence of a male display that had heretofore been undescribed and surprisingly was not performed in all successful courtships. This report describes the courtship of *N. iole* with an emphasis on this new display and the contexts in which the display occurs in the hope of discovering something of its proximate and ultimate functions.

Methods

The dainty sulfur was studied at the Archbold Biological Station 13 south of Lake Placid, Florida, from July to November, 1981. There it flies all year and is most common where *Bidens pilosa* Powell and Turner, its larval foodplant and an adult nectar source, is abundant.

Virgin females were obtained by rearing from eggs. The eggs were collected on sprigs of *B. pilosa* that had been placed in small (8 x 8 x 8 cm) wire cages with field-caught females. The cages were placed outside in full

sun to induce females to oviposit. The larvae were fed on fresh cuttings of
B. pilosa in a laboratory where the light-dark regimen and humidity were
variable and the temperature ranged from 27-29°C.

To observe courtship behavior, particularly that which preceded copula-
tion, virgin females that varied in age from freshly emerged to no more than
3 days of age were released near free-flying males in the field. (Females not
used on day of emergence were stored at 4°C after their wings hardened
until use.) Durations of courtships leading to copulation were measured
with a stopwatch and represent the time from when the male first arrived
(within 2 cm of the female) until the pair had coupled and the male stopped
moving. Written records of the sequence of events observed were also
made. I especially noted (1) whether the female was flying or perched when
the male first approached, (2) whether the female performed a flutter
response or mate refusal posture when alighted, (3) whether the male
performed a wing spread display, (4) any perch changes by the female after
alighting, and (5) whether the male had his wings spread when attempting
copulation. When the wing spread display was observed the relative
positions of the male and female were noted. Details on the criteria used to
judge the occurrence of these behavior patterns are given in "Results."

Courtships staged within outdoor cages were recorded on film using
techniques described by Rutowski (1978 and 1979). In spite of numerous
attempts, I was not successful at recording a wing spread display on
motion picture film, hence detailed information on its temporal structure
was not acquired.

In both the field and the cages, mating pairs were separated within a
minute of coupling so that the female could be used again in observations
of successful courtship. Normal copulation lasts about 15 to 20 min.
Throughout this report "virgin female" refers, in addition to the obvious,
to females that have been previously but briefly coupled with a male.

Throughout the paper parametric summary statistics will be presented
as: mean ± one standard deviation (sample size). The 0.05 level was used
in making all decisions regarding statistical significance.

Results

A. Field Observations: Components of Successful Courtship

Fifty-four successful courtships (= leading to copulation) were observed
in the course of releasing 33 virgin females near males in the field. In the
descriptions and data that follow no female accounts for more than 3
successful courtships. The most typical sequence of events was as follows.

After release and while still flying, the female was approached by the
male. The female then dropped quickly to the ground and alit on a leaf,
grass blade, or the soil. While the female sought out a perch the male either
followed her closely or, in some cases, hovered about 10 to 20 cm over the
female. Once the female had perched, she did not move except to extend

her abdomen out from between the hindwings. The male quickly alit next to the female and oriented head-to-head and tail-to-tail with the female. He then curled his abdomen toward the female's, and brought his genitalia into contact with hers which marked the beginning of copulation. This sequence of events was observed in 24% of the successful courtships observed (Table 1).

TABLE 1

The frequency of occurrence of various types of *N. iole* courtships among the 54 successful courtships observed in the field during the study.

Female initially	Rejection response	Copulation attempt with wing spread	Wing spread display	Percent of all courtships	Duration (sec) [X ± SD (n)]
Perched	−	−	−	12	5.33 ± 1.63 (6)
	−	−	+	5	13.66 ± 6.11 (3)
	−	+	−	11	5.20 ± 1.48 (5)
	−	+	+	0	—
	+	−	−	11**	9.16 ± 4.75 (6)
	+	−	+	9**	13.60 ± 6.43 (5)
	+	+	−	4*	9.00 ± 1.41 (2)
	+	+	+	2	13 (1)
Flying	−	−	−	24	9.70 ± 5.29 (10)
	−	−	+	0	—
	−	+	−	7	14.30 ± 6.13 (4)
	−	+	+	2	24 (4)
	+	−	−	4**	14.50 ± 0.71 (2)
	+	−	+	4	23 (1)
	+	+	−	4	8 (1)
	+	+	+	0	—

*/**Data includes 1 (*) or 2 (**) courtships in which the female changed perch.

Variations in this basic pattern arose when: (1) the female was already perched when the male approached to within 2 cm, (2) the female performed a flutter response or a mate refusal posture when perched, (3) the male performed a display hereafter referred to as the wing spread display, and/or (4) the male attempted copulation with his wings noticeably spread to an angle of about 120-150 degrees. The frequency of occurrence of courtships with these components is given in Table 1. A flutter response (Obara and Hidaka, 1964) was recorded any time a perched female performed at least one wing flick [= a rapid opening and closing of the wings (Rutowski, 1978)] while the male courted her. The mate refusal posture (Obara, 1964) was recorded when, in response to the male's presence, a female assumed a posture with her wings spread and her abdomen raised so that its long axis stood perpendicular to the plane of the female's wings. Rarely (n = 7), females changed their perch after alighting by resuming flight. In subsequent analyses these perch changes were grouped with the flutter response and mate refusal postures as initial rejection responses. The data reveal that perched females were no more likely to perform rejection responses than flying females ($\chi^2 = 2.68$, 1df, p < 0.1).

The wing spread display of the male (Fig. 1) was recorded when, after the female had perched, he alit facing away from the female but no more than 3 cm in front of her. Once on the substrate, the male spread his wings and held them fully opened for several seconds during which time the wings quivered slightly. Also, during the time the wings were spread the forewings were held a little forward of and above the hindwings, enough to clearly expose the red-orange sex brand on the dorsal surface of the male's hindwing (Klots, 1951). Wing spread displays were performed in 22% of the observed courtships. They were also observed in some unsuccessful courtships. In some but not all courtships without the wing spread display, the female alit on a vertical grass blade, in dense vegetation, or in some other location that made it impossible for the male to perform a wing spread display due to the lack of suitable substrate. However, it was not possible to quantify the frequency of this occurrence because of difficulty in establishing exactly what constitutes suitable substrate for a wing spread display.

Whether the female is initially flying or perched has no effect on the likelihood that the male will perform a wing spread display (Table 1). Thirty percent of the perched females and 12.5% of those flying when intially approached by males, were courted with wing spread displays. The difference was not significant ($\chi^2 = 2.36$, 1 df, p = 0.12). However, if the female performed a rejection response (includes perch changes by female during courtship), a male was significantly and almost 4 times more likely to perform a wing spread display. Of the rejecting females, 40% elicited wing spread displays from the courting males; of females that did not, only
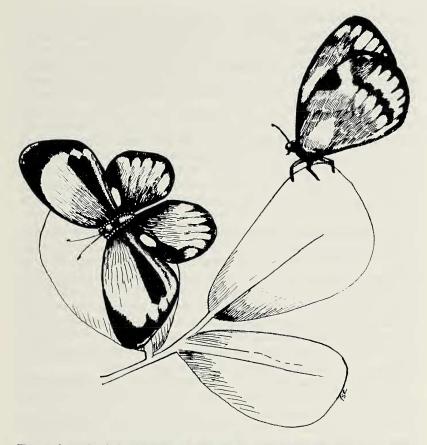
Fig. 1. Courtship behavior in the dainty sulfur, *Nathalis iole*. The male (wing span
         $\sim$ 2.3 cm) has landed in front of and facing away from the female and is
         performing the wing spread display. This figure was drawn from a 35 mm
         photograph.

10.8% elicited the display ($x^2 = 5.81$, 1 df, p = 0.02). The performance of a
wing spread display had no effect on the likelihood that a male would
subsequently attempt copulation with his wings spread ($x^2 = 1.24$, p =
0.26). Males that were accepted in copulation by females were examined
and assessed with respect to forewing length and wing wear. Males that
performed wing spread displays were not different from those that did
with respect to these two characters. Virtually all males were in fresh
condition (more than 80% in both groups) and had forewing lengths (base
to wing tip) of 14 mm [males that performed wing spread display: 14 ± 1
mm (9); those that did not: 13.8 ± 0.62 mm (34)].

B. Field Observations: Temporal Structure of Successful Courtship

Table 1 shows that the duration of successful courtship in *N. iole* is affected by its form. Initial observations indicated that the position of the male's wings (spread or not spread) during copulation attempt had no effect on the duration of the courtship. To test this hypothesis, I compared courtships that varied with respect to the male's wing position during copulation attempt but in which the female did not perform a rejection response, was initially perched and remained so, and was not courted with a wing spread display. The duration of courtships in which the male did not spread his wings during the copulation attempt [5.33 ± 1.63 sec (6)] was not significantly longer than those in which the male did spread his wings [5.2 ± 1.48 sec (5); t = 0.14, 9 df, p = 0.45]. In all analyses presented below, data for courtship with and without this male behavior pattern were pooled.

As a baseline duration I used the mean duration of successful courthsips in which the female (1) was intially perched, (2) performed no rejection responses, and (3) was not courted with a wing spread display. This baseline duration was 5.27 ± 1.49 sec (11) and was used to study the effect of variation in form on the duration of courtship. When the female was initially flying but did not perform a rejection response and was not courted with a wing spread display, the duration of courtship [11 ± 5.71 sec (14)] was significantly longer than the baseline duration (t = 3.23, 23 df, p = 0.002). Females flew for about 5 to 6 sec on the average before alighting if not perched when the male first approached.

If an initially perched female performed a rejection response but was not courted with a wing spread display, the duration of courtship [9.13 ± 4.05 sec (8)] was significantly longer than the baseline (t = 2.92, 17 df, p = 0.005). This was also true if one eliminates from successful courthsips with a rejection response those in which the female changed perch after the male's approach. Although the mean duration of this group was a little shorter [7.8 ± 4.27 sec (5)] these courtships were still significantly longer than those without a flutter response or mate refusal posture (t = 1.8, 14 df, p = 0.047). Hence, rejection responses typically increased the duration of courtship by a factor of 1½ to 2.

Male wing spread display also increased the duration of successful courtship. Courtships identical to the baseline courtships, but including male wing spread display had a mean duration of 13.6 ± 6.11 sec (3), which was significantly longer than the baseline duration (t = 4.5, 12 df, p = 0.001). These data indicate that the wing spread display has an average duration of about 8 sec.

C. Film Records

Using 7 lab-reared virgin females, I recorded 16 successful courtships on motion picture film. In the film records, all females were initially perched

(an artifact of the techniques used) but none were courted with wing spread displays. Females performed flutter responses or mate refusal postures in half of the successful courtships recorded. Restrictions on camera movement prohibited the recording of courtships in which the female flew to a new perch. Only 14 of the courtships recorded were complete; all of the analyses that follow are based on information from these 14.

The mean duration of all courtships was 5.03 ± 3.64 sec (14). However, unlike the situation in the field, the duration of courtship without rejection responses [3.93 ± 3.13 sec (8)] was not significantly shorter than that for courtships with rejection responses [6.07 ± 4.01 sec (6); $t = -1.125$, 12 df, $p = 0.14$]. In spite of this difference between field and cage courtships the film records generally confirm the accuracy of the field measurements. Courtships recorded on film without rejection responses by females were identical in form to the baseline courtships and were not significantly different from them in duration ($t = 1.24$, 17 df, $p = 0.12$). Similarly, courtships recorded on film with rejection responses by females were not significantly different in duration from those of identical form measured in the field ($t = 0.425$, 8 df, $p = 0.34$). However, in both comparisons the difference was in the direction of the film records yielding shorter durations than the field records. This is to be expected in light of the inaccuracies inherent in timing such rapid events with a stopwatch.

In these 16 courtships males showed no preference in the side of the female from which they attempted and achieved copulation (male to female's right: 6 courtships; male to female's left: 10 courtships; $\chi^2 = 1.0$, 1 f, $p = 0.32$).

Discussion

Over 70% of the successful *N. iole* courtships observed during the field portion of this study and all courtships recorded on film were basically similar in temporal and sequential structure to that described for several other pierids (Peterson and Tenow, 1954; Rutowski, 1978, 1979; Silberglied and Taylor, 1978; Suzuki, 1977). These courtships were characterized as rapid affairs, lasting a few seconds, in which the male buffets the female with his wings and body, the female extends her abdomen in response, and the male then alights and attempts copulation. However, in the other 22% of the successful courtships reported here, the male behaved in a way that has not been previously described for any pierid; he assumed a stationary posture in front of the female with his wings spread. Because such a display is so different from any prior observation and because it is a facultative part of successful courtship, questions immediately arise concerning its proximate and ultimate functions.

The proximate function of the display appears to be to provide the female with information about the male not garnered by her during the

intial phases of courtship. An initial rejection response by a female
increases the likelihood that the male will perform a wing spread display.
Both the rejection response and the display almost double the duration of
courtship over what it is without them. Hence, females that perform
rejection responses gain time and a display with which to make a more
complete assessment of a potential mate. The exact nature of the signals
involved in this assessment are not known but could involve visual signals
including the lack of ultraviolet reflectance and the black bar on the dorsal
forewing (Rutowski, 1977), and/or chemical signals that probably arise
from the male's sex brand on the dorsal hindwing (Vetter and Rutowski,
1978).

What information is the female gathering about the male during the
display? It seems most likely that the female is assessing the male's (1)
sexual identity, (2) species identity, and/or (3) quality as a mate relative to
other conspecific males. Both female and male pierids are known to
approach and chase conspecifics on occasion (Rutowski, 1980; Rutowski
et al., 1981). Several observations were made during this study of *N. iole*
females engaging in such behavior. The wing spread display may be a way
for females to confirm the sex of the courting animal by the performance of
the display and by visual and chemical signals enhanced by the display.
Information about the species identity of a courting male may also be
important to the female in that *N. iole* is sympatric over a large part of its
range with *Eurema daira* Godart and *E. lisa* Boisduval and LeConte, two
species of small sulfurs closely related and visually and behaviorally
similar to *N. iole* (Rutowski, 1977, 1978). Again, the display may provide
visual, chemical, and behavioral confirmation of a male's species identity.
Finally, the display may provide the female with information about the
male's age, size, persistence, or other characteristics that could be
indicative of his overall genetic quality and ability to invest in the female's
offspring. Male butterflies are known to pass nutrients to the female
during copulation that she may use in oogenesis (Boggs, 1981; Boggs and
Gilbert, 1979; Boggs and Watt, 1981). Rutowski (1982) has reviewed the
Lepidoptera for some of the characteristics of males that may be
important in selection among conspecific males by females.

In summary, the male display appears to have evolved as a way of
delivering information to females who are initially unreceptive. Exactly
which portion of the information presented to the female by the display is
most important to her is as yet unclear. However, by comparing the
behavior of *N. iole* with that of its sympatric and very similar relatives *E.
lisa* (Rutowski, 1978) and *E. daira* (unpubl. data) it may be possible to
evaluate the three hypotheses about its ultimate function proposed in the
previous paragraph.

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